PAN-PACIFIC ENTOMOLOGIST
72(1): 5–12, (1996)

DISCOVERY OF *COLPOCLYPEUS FLORUS* (WALKER) (HYMENOPTERA: EULOPHIDAE) IN APPLE ORCHARDS OF WASHINGTON

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Abstract.—Routine collections of leafroller larvae in Washington apple orchards for the purposes of assessing the kinds of parasites and levels of parasitism revealed the presence of an unknown species. Specimens sent to the USDA-ARS Systematic Entomology Laboratory, Beltsville, were identified as *Colpoclypeus florus* (Walker). This report represents an extension of its geographic range, the first record in the U.S., as well as a new host record. *Colpoclypeus florus* is a gregarious ectoparasitic eulophid which is widely distributed in Europe where it is the most common natural enemy of tortricids in orchards and other crops. It was introduced into Canada and released in small numbers in 1966 and 1967. No recovery was reported until 1992 when it was reared from larvae of obliquebanded leafroller and eyespotted bud moth in Ontario. In that same year the level of parasitism by *C. florus* in two unsprayed apple orchards in Washington was 66% and 75%. The parasite's behavior and key characters that indicate its possible presence in an orchard are discussed. Notes on the distribution in Washington and methods of rearing *C. florus* on pandemis leafroller larvae are described, along with its potential as a biological control agent in Washington.

Key Words.—Insecta, *Colpoclypeus florus*, Eulophidae, leafroller parasite, new host record

Two species of leafroller (Lepidoptera: Tortricidae), *Pandemis pyrusana* Kearfott, the pandemis leafroller (PLR), and *Choristoneura rosaceana* (Harris), the obliquebanded leafroller (OBLR), are the most common pests of pome fruit in Washington State (Beers et al. 1993; Brunner 1984, 1991). Both species overwinter as a second or third instar larva and complete two generations per year (Brunner & Beers 1990). Insecticides are the primary tactics used to control these pests (Brunner 1988). Natural enemies have been reported attacking leafrollers in the western United States. In Washington, however, the natural suppression of leafrollers has generally not been sufficient to provide commercially acceptable control. This is probably due to the use of broad-spectrum insecticides used to control codling moth, *Cydia pomonella* (L.), that are toxic natural enemies of leafrollers.

This is the first report of the parasite, *Colpoclypeus florus* (Walker), attacking PLR in Washington apple orchards. It represents an extension of the geographic distribution of *C. florus,* as well as a new host record.

MATERIALS AND METHODS

Third through fifth instar PLR and OBLR were collected from several apple orchards near Wenatchee, Washington, and Milton-Freewater, Oregon, in the spring of 1992 (Table 1). Potential leafroller feeding sites, one or more leaves or flower parts held together with webbing, were collected, placed in plastic food storage bags and returned to the laboratory. Feeding sites were examined and healthy larvae were placed individually in one-ounce plastic portion cups (Prairie Packaging, S-100), along with a cube of modified pinto bean diet (Shorey & Hale 1965). If parasite larvae or pupae were found, they were placed into a cup along with the remains of the host and leaf. Cups were kept at a constant temperature

(23° C) and a long photoperiod (16:8, light : dark) until larvae died or adult leafrollers or parasites emerged.

In late July PLR larvae of a second summer generation were collected from orchards in Wenatchee (Table 1). Some PLR larvae were attacked by an unknown ectoparasite. The webbing of the rolled leaf shelter of larvae attacked by this parasite was uncharacteristically tough. When such sites were found, the entire leaf, or the portion containing the parasitized leafroller larva, was placed in a small tight-sealing plastic petri dish (Falcon 1006—50 × 9 mm) which was then placed in a food storage container. When an apparently healthy larva was found, it was placed in a one-ounce plastic portion cup with a cube of the modified pinto bean diet. All cups were held in the conditions described above and were examined two times per week until a parasite or an adult leafroller emerged. Any parasites emerging from leafroller larvae or pupae were placed in glass vials with alcohol and labeled as to host-collection site and date. Adults of the ectoparasitic eulophid not previously reared from PLR were sent to the USDA-ARS Systematic Entomology Laboratory, Beltsville, for identification.

In late August, several unsprayed orchards were sampled for the density of leafroller larvae. Twenty shoots, on 40 to 60 trees, were examined for presence of larval feeding sites. Feeding sites were opened and a record was kept of those which were parasitized by the eulophid.

Adults of the ectoparasite which emerged from field-collected PLR larvae were counted and sexed. One male and one female were placed inside a one-ounce cup along with a fourth instar PLR larva. Each cup contained a cube of bean diet as a food source for the leafroller, and honey was streaked on the top or side of the cup as a carbohydrate source for the parasites. Cups were placed inside a food storage container and kept at constant temperature 23.9° C and long photoperiod (16:8, light:dark). Cups were examined three times per week to determine the status of leafroller and parasites. The number of leafrollers which emerged as adults and the number of parasites emerging from each leafroller larva were counted and sexed. The size of each life stage of *C. florus* was determined by measuring the length and width of 30 individuals of each stage, randomly selected from the parasite colony maintained in the laboratory, using a ocular micrometer and dissecting microscope.

RESULTS AND DISCUSSION

The ectoparasitic eulophid reared from PLR larvae was identified as *C. florus* (M. E. Schauff, Systematic Entomology laboratory, USDA, Beltsville). The discovery of *C. florus* attacking larvae of PLR in Washington represents the first record of the species in the United States, as well as a new host record. *Colpoclypeus florus* was transported to Ontario, Canada, from France and Italy and released against the strawberry leafroller, *Ancylis comptana fragariae* (Walsh & Riley), in 1966 and the redbanded leafroller, *Argyrotaenia velutinana* (Walker), in 1967. However, no record of its recovery was ever noted (Williamson 1966, Hikichi 1971). Hagley & Barber (1991) reported rearing *C. florus* from OBLR and *Spilonota ocellana* (Dennis & Schiffermüeller), the eyespotted bud moth. This report represented the first official recovery of *C. florus* in Canada.

Colpoclypeus florus is a gregarious ectoparasite of tortricids and is often the most common and important natural control in European orchards and other

Table 1. Site descriptions where leafroller larvae were collected for determining parasitism levels.

Site	Location	Crop	Management type
TFREC	Wenatchee	apple	unsprayed
Birchmont	Wenatchee	apple	soft pesticide/unsprayed
Marley	Yakima	apple	conventional
WSU	Pullman	apple	feral trees
Davis	Milton-Freewater	apple	conventional/non-bearing
BF	Milton-Freewater	apple	semi-abandoned
Baneck	Milton-Freewater	apple	conventional
Wondra	Milton-Freewater	apple	abandoned

crops (Evenhuis 1974a, Evenhuis & Vlug 1983, Gruys & Vaal 1984, Limon & Blasco 1973, Monta & Gambaro 1973, Scaramozzino & Ugolino 1978, van Veen and & van Wijk 1987, Vives 1980). *Colpoclypeus florus* parasitizes larvae of several tortricid species in Dutch apple orchards and survives control programs based on insect growth regulators (Gruys & Vaal 1984, de Reede et al. 1984, Helsen & Blommers 1989). In Germany *C. florus,* along with *Meteorus ictericus* (Nees) and *Apanteles ater* (Ratzburg), parasitized up to 80% of *Adoxophyes orana* (Fisher von Röslerstamm) in apple orchards treated with the insect growth regulator fenoxycarb (Harzer 1990). In Italian orchards, *C. florus* was responsible for 95% parasitism of *Argyrotaenia pulchellana* Haworth (Monta & Gambaro 1973).

Colpoclypeus florus is not well synchronized with most tortricid species found in European apple orchards. It is most common in mid- to late summer when it parasitizes a high percentage of tortricid larvae (Gruys & Vaal 1984). However, it is present in low levels in the spring and does not appear to overwinter on *A. orana,* its most common host in apple orchards (Evenhuis 1974b). European orchard fruit tortricid pests overwinter as young larvae or eggs (Alford 1984), unsuitable host stages for *C. florus. Colpoclypeus florus* probably uses overwinter hosts that occur outside the orchard, such as *Syndemis musculana* (Hübner) which overwinters as full-grown larvae and may provide an important overwintering reservoir for *C. florus* (Blommers et al. 1988). The most important leafroller pests of pome fruit in Washington overwinter as eggs or young instar larvae (Beers et al. 1993). As in Europe, a lack of suitable overwintering hosts may represent a barrier to the survival and consistency of *C. florus* populations in the Pacific Northwest.

How *C. florus* was transported to the western U.S. and why it was not detected until 1992 are unknown. *Colpoclypeus florus* was probably established in British Columbia by the mid-1970s, based on the inclusion of *Colpoclypeus* sp. in a list of parasites reared from leafroller larvae as part of a research project under the direction of Dr. H. F. Madsen (Jerry Vakenti, pers. comm.). Unfortunately this information has not been published. Because the genus *Colpoclypeus* was not known from North America until it was introduced into Canada (Williamson 1966, Hikichi 1971), it is likely that this unpublished report represents the first indication of the presence of *C. florus* in western North America.

Leafroller Parasitism.—Low levels of leafroller parasitism, 0 to 13%, were found in all orchards in spring (Table 2) and no *C. florus* were detected. Summer parasitism levels were higher, especially in two unsprayed apple blocks, TF-5 and

Table 2. Leafroller larvae collected from different sites in 1992 and the results of rearing to determine levels of parasitism.

Locations: Spring 1992 Site ID	# larvae	PLR/OBLR adults	Number of parasites reared				% total parasitism
			C. florus	*Apanteles*	Tachinidae	Other	
PLR sites							
WSU-TFREC-21	86	75	0	0	0	0	0.0
WSU-TFREC-5	100	74	0	0	0	1	1.3
WSU-TFREC-24	87	75	0	0	0	0	0.0
Birchmont-4F east	92	89	0	1	0	0	1.1
Birchmont-4F west	51	47	0	0	0	0	0.0
Birchmont-1A	73	64	0	1	0	0	1.5
Marley	63	48	0	0	0	4	7.7
OBLR sites							
WSU-Pullman	100	72	0	0	0	0	0.0
Davis	100	84	0	0	0	0	0.0
BF	31	22	0	0	0	3	12.0
Baneck[a]	100	41	0	0	0	0	0.0
Wondra	37	17	0	0	0	2	10.5

Locations: Summer 1992 Sites ID	# larvae	PLR adults	Number of parasites reared				% total parasitism
			C. florus	*Apanteles*	Tachinidae	Other	
PLR sites							
WSU-TFREC-5	52	11	38	0	0	2	78.4
WSU-TFREC-24a	59	9	40	0	2	0	82.3
WSU-TFREC-24b	109	32	67	0	2	0	68.3
Birchmont-4F west	78	63	0	0	12	0	16.0
Birchmont-1D1	81	46	3	4	11	1	29.2
Birchmont-1D2	71	42	6	6	12	1	37.3

[a] Low survival level of leafroller larvae probably due to use of Bt products in the orchard prior to larval collections.

TF-24, located at the Washington State University Tree Fruit Research and Extension Center, Wenatchee (WSU-TFREC). In these blocks, *C. florus* was responsible for 75% and 78% of the total leafroller parasitism (Table 2). Two collections of PLR larvae were made in block TF-24 three days apart. The first indication that a different parasite was present in the WSU-TFREC blocks was the discovery of abnormally dense and much tougher webbing than is typically formed by PLR larvae. The webbing made by parasitized leafroller larvae was similar in density to spider webbing, though not of the same consistency.

An average of 13.5 *C. florus* adults was reared from parasitized PLR larvae (Table 3). The sex ratio favored females, 71.8%, as found in Europe (Evenhuis 1974b, Gruys & Vaal 1984, van Veen & van Wijk 1987). *Colpoclypeus florus* was also reared on PLR larvae originating from a laboratory colony reared on artificial diet. The average number of *C. florus* progeny produced from laboratory-reared PLR larvae and their sex ratio were no different from field collected, parasitized PLR larvae (Table 3). *Colpoclypeus florus* is easy to rear on PLR or OBLR larvae in the laboratory, and thus it may be a promising agent for mass rearing and augmentative release for biological control of leafroller pests in Washington orchards.

Table 3. Data on *C. florus* reared from PLR larvae collected from two unsprayed apple orchards in Wenatchee and produced in the laboratory using PLR larvae from a colony.

			Colpoclypeus florus		
Year	Source	No. of leafroller larvae	% male	% female	Avg. no. progeny (adults ± SE)
1992	Field	85	28.2	71.8	13.5 ± 1.02
1992/3	Lab (PLR)	53	27.0	73.0	12.1 ± 1.27

Distribution.—*Colpoclypeus florus* was detected parasitizing PLR larvae at low levels in two commercial orchards, Birchmont-1D1 and 1D2, located 5 km north of the WSU-TFREC orchards. These orchards were treated with conventional insecticides in the pre-bloom period but did not receive additional insecticide treatments during the summer. *Colpoclypeus florus* was also found in an organic orchard in East Wenatchee, 10 km east of the WSU-TFREC location, and in an unsprayed apple orchard 16 km north of Wenatchee along the Columbia River. The abundance of *C. florus* at these sites was very low; only one or two parasitized PLR larvae were found, but the finds indicate a rather widespread distribution of *C. florus* in north-central Washington.

Additionally, *C. florus* was observed by an agricultural consultant (Steve Harris, pers. comm.) in an organic orchard near Brewster, Washington, 100 km north of Wenatchee. Leaves containing pupae from which *C. florus* had already emerged were submitted to the WSU-TFREC for identification. Although this report does not confirm *C. florus* presence in the Brewster orchard, no other ectoparasitic eulophids attack leafrollers in Washington. The OBLR population in the Brewster organic orchard had been very high in the spring, and control measures were planned against the summer generation. However, when searches of growing shoots in July and August revealed high levels of parasitism the grower decided not to apply control sprays.

Parasite Description.—The adult parasite has a shiny black head and thorax (sometimes appearing greenish under reflected light) with a lighter-colored abdomen (Fig. 1A). The average length (head to tip of abdomen) of the female is 1.82 ± 0.01 mm (mean ± SEM). The ventral side of the abdomen is a light cream to golden-brown color. There are two small dark spots in the center and about one-third of the way in from the tip of the abdomen that denote the base of the ovipositor (Fig. 1C). The legs are golden brown. The male *C. florus* is slightly smaller than the female, 1.46 ± 0.02 mm, and has a darker and less ovate abdomen. Eggs are translucent when first laid but later turn a creamy white. They are elongate, slightly curved and average 0.32 ± 0.003 mm in length and 0.08 ± 0.001 mm in width (Fig. 1B). Neonate larvae migrate to the host larva and begin feeding. *Colpoclypeus florus* larvae are a bright green when young, turning to a pale green or cream color as they near maturity (Fig. 1D). Mature larvae average 1.90 ± 0.04 mm in length and 0.67 ± 0.01 mm in width. When mature, *C. florus* larvae leave the host larva but remain within the host's webbed shelter to pupate. Pupae are naked and a light brown color which soon darkens. Female pupae are slightly more ovate than males, averaging 1.94 ± 0.03 mm in length and 0.78 ± 0.01 mm in width (Fig. 1E). Male pupae average 1.60 ± 0.02 mm in length and 0.62 ± 0.01 mm in width.

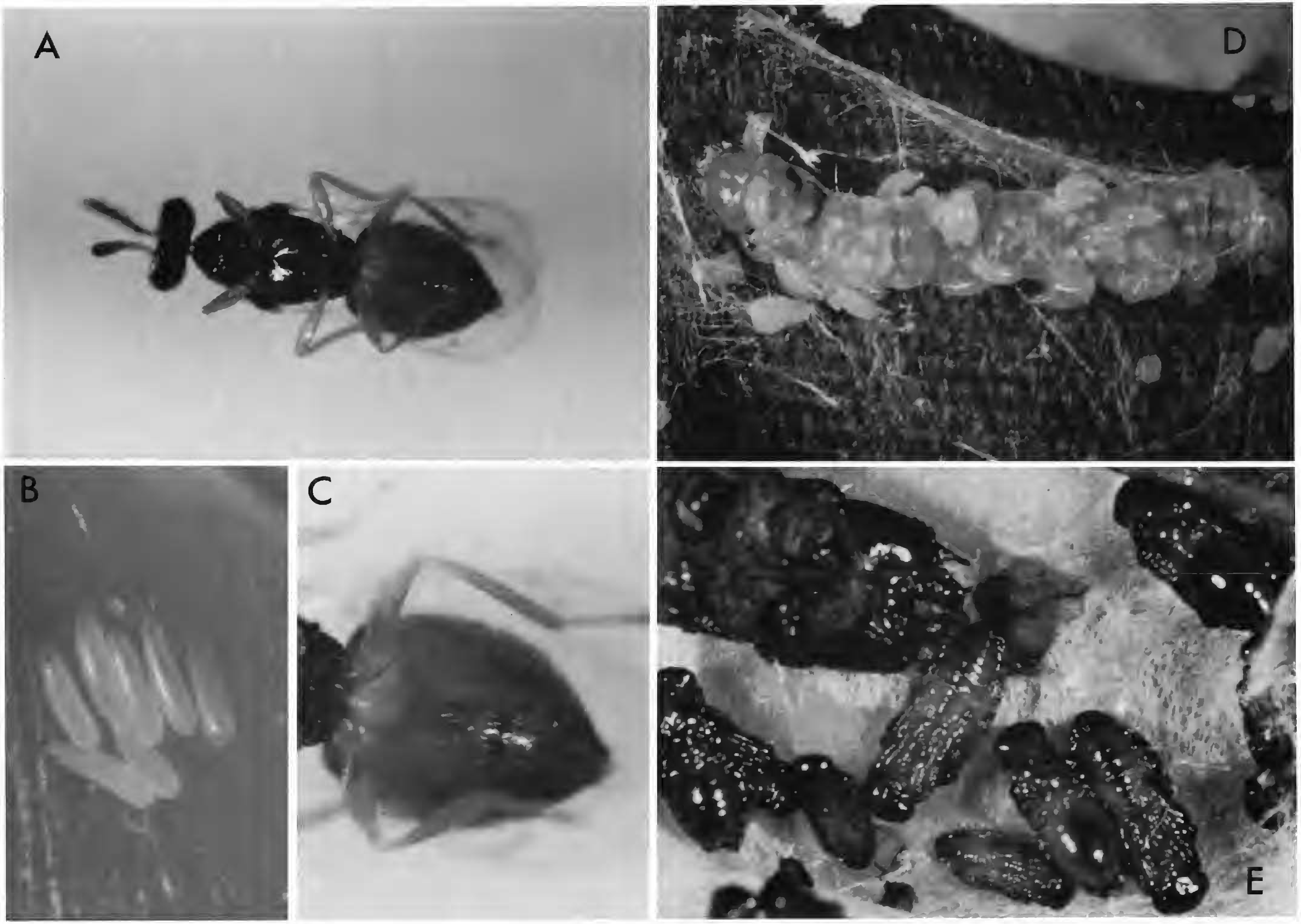

Figure 1. Life stages of *Colpoclypeus florus* (Walker); A—adult female, B—eggs, C—female abdomen ventral view, D—larvae feeding on leafroller, E—pupae.

Parasite Behavior.—My observations confirm that *C. florus* does not paralyze its host (van Veen & van Wijk 1987). Males typically emerge first and mate with females as they emerge. In my laboratory, adults usually remained within the host's webbed shelter for two to three days until all had emerged, and then they all departed over a short period. I observed female *C. florus* make rapid thrusting movements with the ovipositor in the direction of the head capsule of a leafroller larva. However, I have never observed the ovipositor penetrate the caterpillar's head capsule. After the attack there was significant change in the behavior of the leafroller. It stopped feeding, began spinning a dense webbed shelter, and often voided fluids. The *C. florus* female remained with the leafroller larva within the densely webbed shelter and deposited eggs.

The presence of *C. florus* in Washington provides another natural enemy to combat leafrollers. It has been reared on both PLR and OBLR in my laboratory and may be a parasite that could be mass reared for timely releases. The unusually high levels of leafroller parasitism noted in unsprayed orchards suggests that *C. florus* is well adapted to the eastern Washington climate. The susceptibility of *C. florus* to conventional insecticides in Europe suggests that it may be most useful as a biological control of leafrollers in fruit orchards using alternative controls for key pests, such as insect growth regulators, pheromones or bacterial insecticides.

Acknowledgment

I thank M. E. Schauff, Systematic Entomology laboratory, USDA, Beltsville for the rapid identification of specimens submitted and Drs. Bill Turner, Elizabeth Beers and Rich Zack for critical reviews and suggestions which helped improve the manuscript. I am especially grateful to the able assistance of Mike Doerr and Lisa Mill for their technical skills in rearing leafrollers and parasites in my laboratory. I acknowledge the invaluable contribution of Washington's fruit growers who partially funded this research through the Tree Fruit Research Commission.

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